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Embryonic Origins of a Motor System: Motor Dendrites Form a Myotopic Map in *Drosophila*

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The organisational principles of locomotor networks are less well understood than those of many sensory systems, where in-growing axon terminals form a central map of peripheral characteristics. Using the neuromuscular system of the *Drosophila* embryo as a model and retrograde tracing and genetic methods, we have uncovered principles underlying the organisation of the motor system. We find that dendritic arbors of motor neurons, rather than their cell bodies, are partitioned into domains to form a myotopic map, which represents centrally the distribution of body wall muscles peripherally. While muscles are segmental, the myotopic map is parasegmental in organisation. It forms by an active process of dendritic growth independent of the presence of target muscles, proper differentiation of glial cells, or (in its initial partitioning) competitive interactions between adjacent dendritic domains. The arrangement of motor neuron dendrites into a myotopic map represents a first layer of organisation in the motor system. This is likely to be mirrored, at least in part, by endings of higher-order neurons from central pattern-generating circuits, which converge onto the motor neuron dendrites. These findings will greatly simplify the task of understanding how a locomotor system is assembled. Our results suggest that the cues that organise the myotopic map may be laid down early in development as the embryo subdivides into parasegmental units.

Introduction

The way in which neural networks underlying locomotion are specified and assembled is less well understood than the development of other parts of the nervous system, particularly the sensory nervous system. One of the reasons for this is that, in many sensory systems, in-growing sensory axons are marshalled to form a clear anatomical map of peripheral characteristics in the central nervous system (CNS) (for reviews, see Knudsen 2002; Keller and Vosshall 2003; McLaughlin et al. 2003). This straightforward anatomical outcome of the developmental process, which rather explicitly reflects the function of the neurons concerned, means that developmental observations and experiments can readily be interpreted in terms of axon growth and targeting within the orderly framework of the map. For motor systems, on the other hand, there appears to be no such simplifying anatomical correlate of function, at least insofar as the underlying patterns of neuronal connectivity are concerned. Nonetheless, there are some regularities on the motor side. In the vertebrate spinal cord, motor neurons are organised into pools and columns that form a neural correlate of the anatomy of the body musculature they innervate. Motor neurons innervating the same muscles are clustered into pools, and motor pools are grouped into columns, each supplying a different muscle set (Landmesser 1978; Tsuchida et al. 1994). The organisation of motor pools is highly conserved among different species and, to a degree, reflects the distribution of the muscles that they supply (Romanes 1951; Cruce 1974; Landmesser 1978). However, motor pools (and columns) reflect the locations of motor neuron cell bodies, but not the regions of the spinal cord where their dendritic arbors receive synaptic connections. Thus, it is not clear whether motor columns are simply a consequence of the process by which motor neurons are generated and specified or whether they actually reveal an underlying functional organisation in the motor system (Landmesser 1978). The task of understanding how the system is assembled would be greatly simplified if an underlying principle to the organisation of connectivity in a motor system could be demonstrated.

We decided to investigate this question in a relatively simple, genetically amenable motor system, that of the *Drosophila* embryo and larva. The *Drosophila* neuromuscular system is advantageous for several reasons. The larval motor system generates a simple, quantifiable output in the form of peristaltic waves of muscle contractions towards the end of embryogenesis (Siekhaus and Fuller 1999; Suster and Bate 2002), and much is known about how the machinery that

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Abbreviations: AC, anterior commissure; aCC, anterior corner cell; AEL, after egg-laying; CAM, cell adhesion molecule; CNS, central nervous system; DA, dorsal acute; DiD, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine perchlorate; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; DO, dorsal oblique; DT, dorsal transverse; *en*, *engrailed*; *eve*, *even-skipped*; fpCC, friend of pCC; *gcm*, *glial cells missing*; *gsb*, *gooseberry*; h, hours; *hh*, *hedgehog*; HRP, horseradish peroxidase; ISN, intersegmental nerve; LL, lateral longitudinal; LT, lateral transverse; NB, neuroblast; *nkd*, *naked*; PC, posterior commissure; pCC, posterior corner cell; *ptc*, *patched*; SBM, segment border muscle; SN, segmental nerve; TN, transverse nerve; VA, ventral acute; VL, ventral longitudinal; VNC, ventral nerve cord; VT, ventral transverse; VUM, ventral unpaired median; *wg*, *wingless*

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executes this behaviour (consisting of 30 muscles per abdominal half-segment and approximately 36 innervating motor neurons) develops (Bossing and Technau 1994; Landgraf et al. 1997; Schmidt et al. 1997; Ruiz-Gómez 1998; Schmid et al. 1999). The system is amenable to electrophysiological techniques (Baines and Bate 1998) and lends itself to experimental analysis, as most of its elements can be genetically manipulated with relative ease and specificity (Baines et al. 1999, 2001, 2002; Suster and Bate 2002).

We have focused on those elements of the system that have already been identified and to which we can readily gain access—i.e., the motor neurons and the body wall muscles that they innervate. What we find is a clear organisational principle, namely that motor neuron dendrites (rather than their cell bodies) are partitioned in such a way that their positions in the neuropile correlate with the distribution of their respective target muscles. Thus, motor neuron dendritic fields are organised as a myotopic map, which represents centrally the array of body wall muscles in the periphery. Since the motor neuron dendrites are necessarily the structures on which the outputs of the central pattern-generating circuits will be distributed, this myotopic map reveals a first layer of organisation to the underlying connectivity of the motor system.

We have addressed two questions related to this map: the way in which the central representation is organised and the sorts of mechanisms that might regulate its development. Our results suggest that the formation of the myotopic map is an active process of dendritic growth and elaboration, rather than a passive consequence of the way the motor neurons are generated and packed within the CNS. We also show that the mapping process is autonomous to the CNS and not imposed by contact with the muscles themselves. The map is repeated in a parasegmental fashion along the CNS, which leads us to believe that boundaries in the CNS and the cues that organise the map may be established as a result of events early in development as the embryo subdivides itself into a series of parasegmental units.

Results

Organisation of the Larval Motor System

We began our analysis by correlating the positions of motor neuron dendrites with the distribution of their muscle targets in the periphery. We retrogradely labelled motor neurons in a pairwise fashion and mapped the positions of their dendritic arbors. Because our interest lies in the mechanisms that underlie the assembly of the motor system, we focused on stages when each motor neuron first establishes a characteristic domain of arborisation within the neuropile (early stage 17, 15h after egg-laying [AEL]).

Motor axons project into the muscle field via two main nerves, the intersegmental (ISN) and the segmental nerve (SN) (Bate 1982; Thomas et al. 1984; Landgraf et al. 1997). The transverse nerve (TN) runs along the segment border and has few motor axons (Figure 1A) (Chiang et al. 1994; Gorczyca et al. 1994; Thor and Thomas 1997; Schmid et al. 1999). Choice of nerve root is one of several features that divide the motor neurons into two principal sets, the ISN and SN. First, the cell bodies of SN motor neurons are located in the same segment as the muscles that they innervate, whereas ISN motor neuron somata are located in the segment next anterior (with the exception of the RP2 and two neuromodulatory efferent ventral unpaired median [VUM] neurons; Figure 1A) (Landgraf et al. 1997). Second, ISN motor neurons innervate internal muscles, which span a segment from anterior to posterior, whereas SN (and the TN) motor neurons innervate external muscles. External muscles are distinct from the internal set in several respects: (a) they are generally transverse; (b) unlike internal muscles, they require *wingless (wg)* signalling for their specification (Baylies et al. 1995); (c) external (but not internal) muscles and their innervating motor neurons express the cell adhesion molecule (CAM) Connectin, with the single exception of muscle ventral

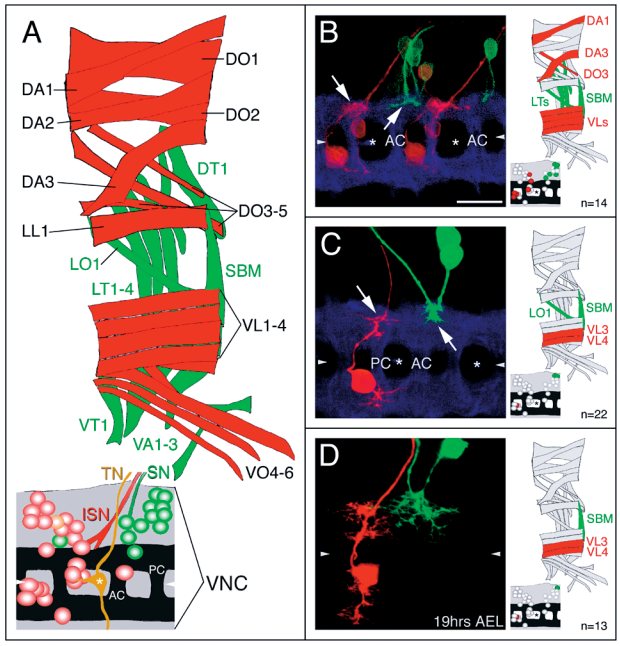

Figure 1. Organisation of the Larval Motor System

(A) Diagrams of abdominal body wall muscles of one half segment (top) and of the VNC (bottom). These diagrams are included in most subsequent figures as reference for the relative positions of muscles and their innervating motor neurons. Internal muscles are red; external muscles, green. Muscle nomenclature is according to Bate (1993): muscle position (D, dorsal; L, lateral; V, ventral), followed by orientation (A, acute; L, longitudinal; O, oblique; T, transverse) and SBM. In the VNC, cell bodies of motor neurons innervating an abdominal half-segment are indicated: red shows ISN with internal muscle targets; green shows SN with external muscle targets. The TN (brown) coincides approximately with the segment boundary. TN exit glia (asterisk) located on the ventral midline are also shown. The neuropile is indicated in black.

(B and C) Retrograde fills of ISN and SN motor neurons in 15-h-old wild-type embryos. The neuropile (blue) was visualised with anti-HRP. ISN and SN motor neurons innervating muscles in the same segment elaborate their dendrites (arrows) in distinct regions. In (B), groups of ISN and SN motor neurons are labelled in two consecutive segments. In (C), individual ISN and SN motor neurons of a segment are shown.

(D) The separation between ISN and SN motor neuron dendrites appears to be maintained, at least until 19 h AEL, when the motor system is functional.

Anterior is left and dorsal is up. Symbols and abbreviations: triangles, ventral midline; AC, anterior commissure; PC, posterior commissure; asterisks, TN exit glia in (A) and dorsoventral channels, which are landmarks for the segment borders, in (B)–(D). Scale bar (not applicable to diagrams of CNS and muscle field): (B) 18 μm; (C–D) 14 μm. *n* numbers refer to the number of segments in which the same types of motor neurons (e.g., RP3 and SBM in [C]) were labelled.

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transverse 1 (VT1) (Nose et al. 1992; Meadows et al. 1994; (Prokop et al. 1996).

In addition, we find that ISN and SN motor neurons elaborate their dendrites in distinct regions of the neuropile (Figure 1B–1D) (Landgraf et al. 1997). Dendrites of ISN motor neurons occupy a domain extending posteriorly from the posterior part of one neuromere into the anterior part of the next. SN motor neuron dendrites occupy a domain that lies between the domains of ISN motor neuron arbors (Figure 1B).

Thus, the organisation of the body wall muscles into internal and external sets is reflected centrally in patterns of motor neuron arborisations. The innervating motor neurons project their axons through different nerves and elaborate their dendritic fields in distinct regions of the neuropile. Although dendritic arbors become progressively more elaborate and extensive over developmental time, their separate domains remain clearly recognisable and appear to be maintained at least until the motor system is fully functional (18 h AEL) (Baines and Bate 1998) (Figure 1D).

A Map of the External Muscles

Having established that there is a central representation of the muscle field, we examined the organisation of the motor neuron dendrites in greater detail. We looked first at the set of external muscles and their innervating (SN) motor neurons. Muscles of similar anteroposterior positions, such as the ventral acute muscle (VA3) and the segment border muscle (SBM), are innervated by motor neurons whose dendritic arbors lie in a common region of the neuropile (Figure 2A). Conversely, motor neurons supplying the anterior (lateral transverse 1–2 [LT1–LT2) versus the posterior (SBM) muscles have dendritic arbors that are correspondingly separated in the anteroposterior axis of the CNS (Figure 2B).

To put the idea of a regular map to the test, we focused on an unusual external motor neuron–muscle pair. Muscle VT1 is innervated by a TN rather than an SN motor neuron (Gorczyca et al. 1994; Landgraf et al. 1997). However, VT1 lies at the same place in the anteroposterior axis as the SBM, although VT1 is ventral and the SBM more dorsal. We find that the VT1 motor neuron dendritic field overlaps with that of the SBM motor neuron (Figure 2C). For the external set, we conclude that differences in target muscle location in the anteroposterior axis are mapped centrally as regular differences in dendritic position, but dorsoventral distinctions are not (Figure 3).

A Map of the Internal Muscles

We next asked whether there is a similarly regular representation of the internal muscles in the developing CNS. While most external muscles are transverse and have unique anteroposterior locations, the internal muscles span the width of a segment so that positional distinctions between them are solely in the dorsoventral axis. We find that the set of internal muscles is represented centrally by three dendritic domains. Motor neurons innervating ventral internal muscles elaborate their dendritic arbors in the anterior half of the ISN dendritic domain (see Figure 2D and 2F; Figure 3). Motor neurons with dorsolateral internal muscle targets (lateral longitudinal [LL] 1, dorsal acute [DA] 3, dorsal oblique 3–5 [DO3–DO5]) put their arbors into the posterior part of the ISN dendritic domain (see arrow in Figure 2E; Figure 3). Finally, dorsal muscles are represented by a motor neuron dendritic domain that lies between those representing ventral (anterior) and dorsolateral (posterior) internal muscle groups (see arrowhead in Figure 2E; Figure 2F; Figure 3). Thus, the internal muscles are represented in the neuropile by three domains of dendritic arborisation that reflect their different dorsoventral locations in the periphery. Once again, we can conclude that there is a regular mapping of muscle position in the neuropile: in this case, it is positions in the dorsoventral axis peripherally that are represented centrally as differences in the anteroposterior locations of dendrites.

Atypical Motor Neurons Conform to the Myotopic Map

To test the idea that dendritic arbor positions relate to the distribution of muscles, we looked at an atypical motor neuron–muscle pair. The RP2 motor neuron is reported to innervate dorsal muscle DA2 (Sink and Whitington 1991; Landgraf et al. 1997), yet its dendrites span the domains that represent both dorsal and dorsolateral internal muscles (see Figure 2G). However, on careful analysis we find that DA2 is, in fact, specifically innervated by a U neuron whose dendrites lie in the dorsal internal domain (Schmid et al. 1999) (see Figure 2G), whereas the RP2 axon forms endings generally on all dorsolateral and dorsal muscles by 19 h AEL (see Figure 2H). These seem to correspond to the type 1s boutons found in late larvae (Atwood et al. 1993; Jia et al. 1993; Landgraf et al. 2003). Thus, the RP2 neuron puts its dendrites into a region of the neuropile that does indeed represent its targets, namely the dorsolateral and dorsal internal muscles.

A Parasegmental Organisation of the Neuromuscular System

Like the muscle field itself, the map of motor neuron dendrites is metamerically repeated. However, we find that the boundaries of these two units are out of register with one another, since the dendrites of the motor neurons innervating internal muscles lie in the next anterior neuromere. The anterior border of the dendritic map, as defined by the extent of these anterior dendrites, coincides with the anterior margin of *engrailed* (*en*) expression (Figure 4A). Thus, while the muscles are segmental in their organisation, the domains occupied by the dendrites of their innervating motor neurons are parasegmental.

To test whether genes that implement the parasegmental pattern in the epidermis (Hatini and DiNardo 2001) are also required for the formation of the parasegmental organisation of the neuromuscular system, we studied the formation of SN and ISN dendritic fields in embryos singly mutant for the following segment polarity genes: *en/invected* ($Df(en^{E})$), *wg* (wg^{CX4}), *naked* (nkd^{2}), *patched* (ptc^{9}), *hedgehog* (hh^{21}), and *gooseberry* (*Df2R(gsb)*). Every one of the six different mutants that we have analysed has partially aberrant patterns of neuroblasts (NBs) (Chu-LaGraff and Doe 1993; Skeath et al. 1995; Bhat 1999; Deshpande et al. 2001). Nevertheless, SN and ISN motor neurons still form and can be identified by their characteristic axonal projections into the periphery. In addition, we find that the fundamental separation between SN and ISN dendritic domains is present despite often severe perturbations in CNS structure (Figure 4B–4H). For example, in *gsb* mutant embryos, both nerve roots are frequently fused so that the SN and ISN share a common CNS exit point (Patel et

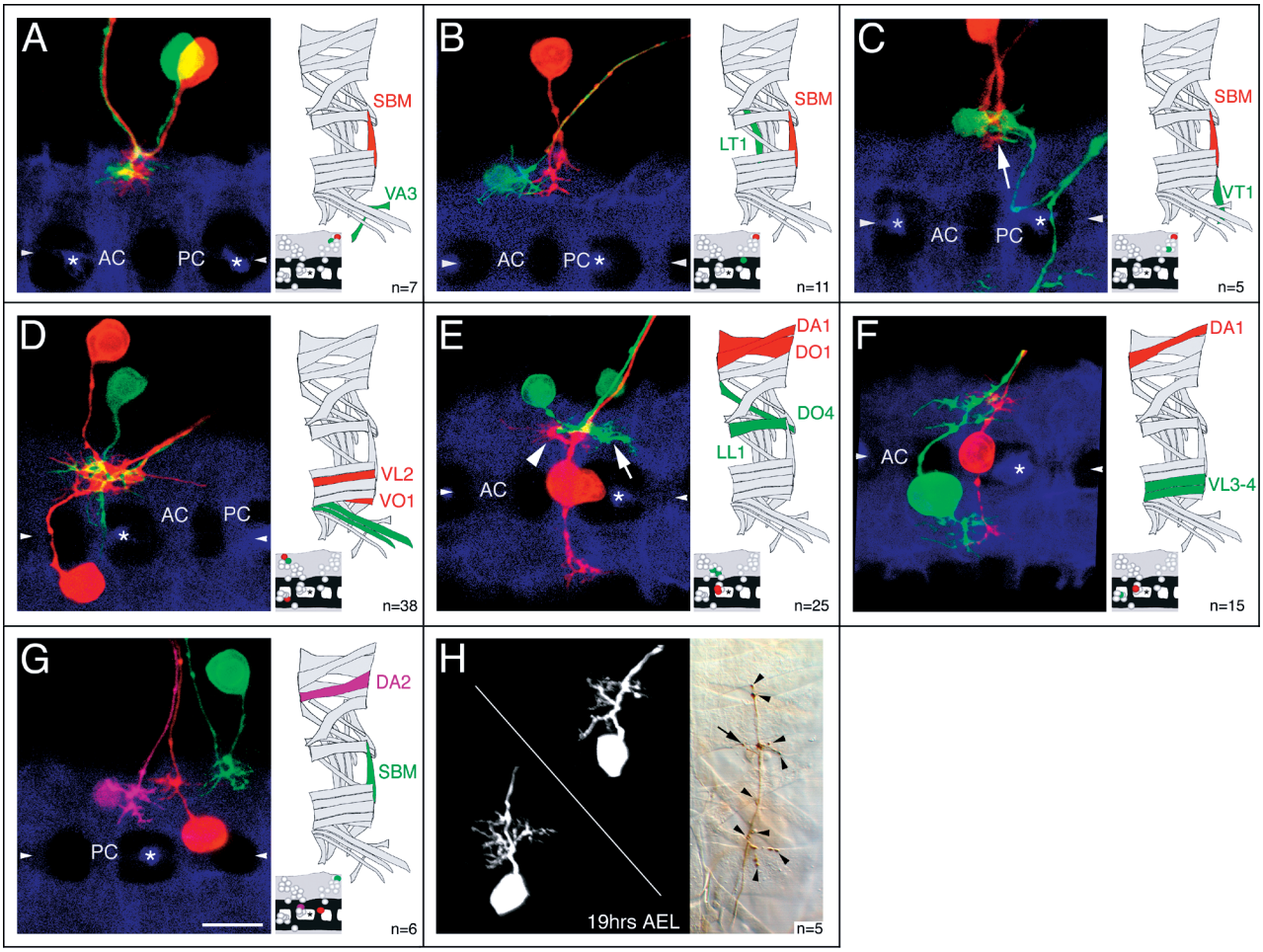

Figure 2. Central Myotopic Representation of the Muscle Field

Motor neurons with external (A–C) and internal (D–G) muscle targets (indicated in muscle diagrams) were retrogradely labelled in 15-h-old wild-type embryos. The neuropile, visualised with anti-HRP, is shown in blue.

(A) Motor neurons with ventral and lateral external muscle targets at a similar location in the anteroposterior axis elaborate their dendritic arbors in a common region of the neuropile, within the SN dendritic domain, which represents external muscles.

(B) Differences in muscle positions in the anteroposterior axis are reflected centrally by corresponding distinctions in the anteroposterior locations of motor neuron dendritic fields.

(C) Muscle VT1 is innervated by a TN motor neuron (and frequently also by its contralateral homologue, partly shown). Dendrites of the VT1 motor neuron (arrow) coincide with those of the SBM motor neuron of the next anterior segment. Note that in (A) the position of the SBM dendritic field is shifted somewhat anteriorly relative to the commissural landmarks as compared to (B) and (C). We observe such shifts relative to the commissural landmarks in 13% ($n = 52$) of SBM (and other) motor neurons at this developmental stage. Importantly, the relative positions of dendritic fields within the myotopic map remain constant. Such shifts relative to the commissures may be linked to the condensation of the nerve cord, which is underway at this stage.

(D) Motor neurons with ventral internal muscle targets elaborate their dendrites in the ISN dendritic domain, which is located in the posterior part of the next anterior segment.

(E) Motor neurons with dorsolateral internal targets put their dendrites (arrow) in the most-posterior part of the ISN dendritic domain, i.e., posterior to the dendritic domain, which represents dorsal internal muscles (arrowhead).

(F) Motor neurons innervating ventral (ventral longitudinal 3–4 [VL3–VL4]; RP3) and dorsal (DA1; aCC) internal muscles elaborate their dendritic arbors in distinct regions of the ISN dendritic domain. Both motor neurons shown are bipolar and each has a second, smaller contralateral (with respect to the target muscle) dendritic arbor that mirrors the distribution of the ipsilateral dendrites.

(G) Muscle DA2 is innervated by the RP2 (red) and a U/CQ neuron (magenta). The RP2 axonal trajectory through the posterior root of the ISN demarcates the boundary between ISN (magenta and red) and the SN (green) dendritic fields.

(H) On the left are two examples of RP2 neurons filled in different 19-h-old embryos with Lucifer Yellow. As at the earlier stages shown in (G), most of the RP2 dendrites project anterior of the axon into the ISN dendritic domains representing dorsolateral and dorsal internal muscles. On the right is a Nomarski micrograph of a Lucifer Yellow-filled RP2 axon in the periphery at 19 h AEL. Swellings (arrowheads), likely neuromuscular junctions, are not specific to muscle DA2 (arrow), but are seen on all dorsolateral and dorsal muscles.

Anterior is left and dorsal is up. Symbols and abbreviations: triangles, ventral midline; AC, anterior commissure; PC, posterior commissure; asterisks, dorsoventral channels (landmarks for the segment borders). Scale bar (not applicable to diagrams of CNS and muscle field and micrograph of muscle field in [H]): 10 μm.

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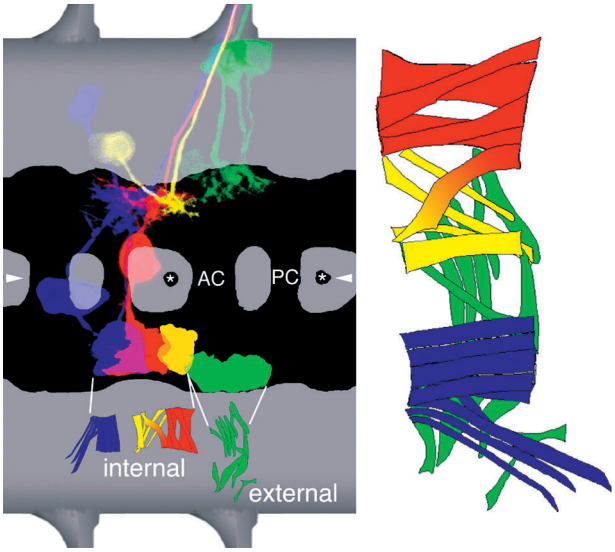

Figure 3. Motor Neuron Dendrites Form a Myotopic Map of the Muscle Field

Collage of superimposed representative motor neurons (of 15-h-old embryos) that innervate the muscles of an abdominal half-segment (shown right). Entire dendritic domains are indicated on the contralateral side. Colour code: blue, ventral internal; yellow, dorsolateral internal; red, dorsal internal; green, external; black, neuropile; grey, cortex. Anterior is left and (for the muscle diagram) dorsal is up.

Symbols and abbreviations: triangles, ventral midline; asterisks, dorsoventral channels (landmarks for the segment borders); AC, anterior commissure; PC, posterior commissure.

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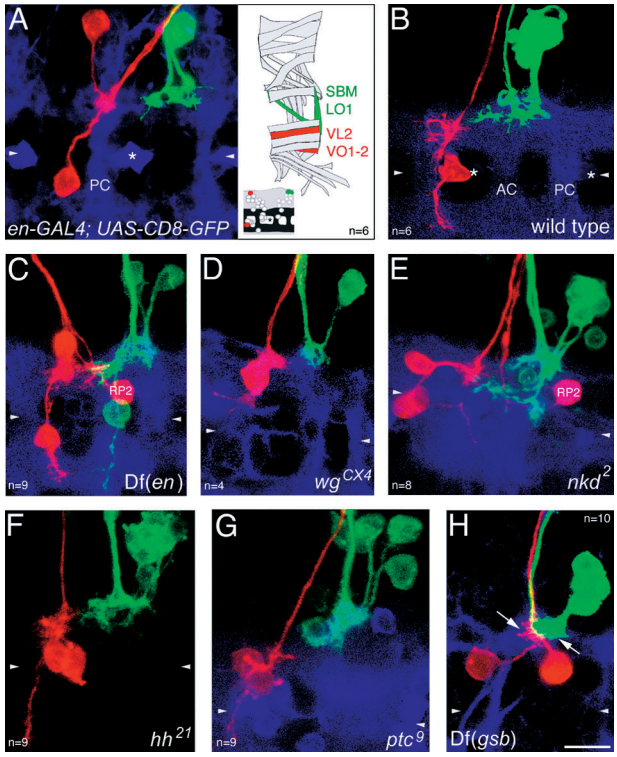

Figure 4. Parasegmental Organisation of the Motor System

(A) Distribution of motor neuron dendritic arbors relative to the domains of *en* expression. Neurons expressing the *en* gene were visualised (blue) using *en-GAL4;UAS-CD8-GFP*. ISN motor neuron dendrites (red) elaborate in the En domain (blue) of the neuromere, whereas SN motor neuron dendrites (green) form in the anterior half of the next posterior segment (asterisks indicate the segment borders). Thus, the motor system appears to be parasegmental in nature. The diagrams to the right indicate which motor neurons were labelled.

(B–H) ISN motor neurons (red) with dorsal internal and SN motor neurons (green) with lateral external muscle targets were retrogradely labelled in 15-h-old wild-type embryos (B) and those mutant for different segment polarity genes (C–H). (C) to (H) should be compared with the wild-type control in (B). As far as could be ascertained, similar sets of motor neurons were labelled in the wild-type (B) and mutants (C–H). The neuropile, visualised with anti-HRP, is shown in blue (except for [F]). In all mutant embryos, with the exception of *Df(gsb)* (H), ISN and SN motor neurons have separate nerve roots and dendritic fields, as in the wild-type. As (H) shows, in *Df(gsb)* mutant embryos, ISN and SN nerve roots are frequently fused, yet the respective dendritic fields (arrows) do not appear to intermingle.

Anterior is left and dorsal is up. Symbols and abbreviations: triangles, ventral midline; AC, anterior commissure; PC, posterior commissure (RP2 cell bodies, the most posterior of the ISN motor neurons, are indicated in [C] and [E]); asterisks, dorsoventral channels (landmarks for the segment borders). Scale bar (not applicable to diagrams of CNS and muscle field): 10 μm.

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al. 1989). Nevertheless, SN and ISN axons as well as their dendritic fields do not intermingle but remain separate (Figure 4H). These results suggest that the subdivision of the neuropile into the principal ISN and SN dendritc domains is a robust feature of the system, which appears to be specified early in development as the embryo subdivides into parasegmental units.

The Myotopic Map Forms Not as a Result of Passive Packing

We next asked what mechanisms underlie the formation of the myotopic map. Because ISN and SN motor neurons lie at different positions in the CNS and their axons grow out into the muscle field through different nerves, it is reasonable to suppose that at least the major subdivision of dendritic arborisations into internal and external domains could be a byproduct of the locations at which the motor neurons are generated and the paths taken by their growing axons. We can exclude this 'passive mapping' explanation by considering a single motor neuron–muscle pair, namely dorsal transverse 1 (DT1) and its innervating motor neuron. DT1 is an external muscle (by position, orientation, *wg* dependence, and Connectin expression), yet its motor neuron is clustered with the internal muscle innervating set and its axon (uniquely for the external muscles) grows out through the ISN. Despite its packing within the 'internal motor neuron' set, the DT1 motor neuron makes a long posterior projection through the internal muscle domain of the myotopic map to reach the external domain, where it arborises appropriately, reflecting the orientation and external nature of its target muscle (Figure 5A). In contrast, motor neurons derived from the same NB as DT1 innervate neighbouring internal muscles DO3–DO5 and put their dendrites in a more anterior region characteristic of the dorsolateral muscles (Figure 5B and 5C) (Landgraf et al. 1997). These findings strongly suggest that the mapping of the muscle field within the CNS is an active process of growth and arborisation that partitions dendrites into subdomains of the neuropile that are appropriate to their function, rather than a passive subdivision of available space by position of origin or axon trajectory.

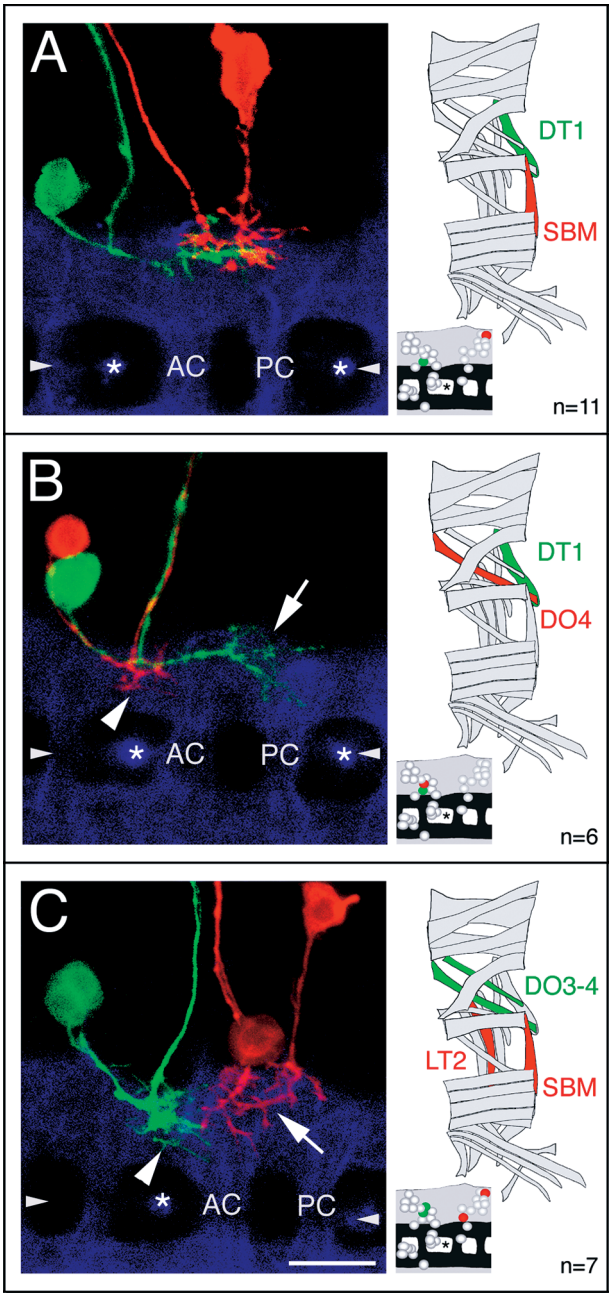

Figure 5. The Myotopic Map Forms by an Active Process of Dendritic Growth and Arborisation

ISN motor neurons (indicated in muscle diagrams) were retrogradely labelled in 15-h-old wild-type embryos. The neuropile, visualised with anti-HRP, is shown in blue.

(A) External transverse muscle DT1 is innervated by an ISN motor neuron (green) whose dendrites overlap with those of the SBM motor neuron (red).

(B and C) Internal muscles DO3–DO5 are innervated by motor neurons derived from the same NB as the DT1 motor neuron, and all have common axonal trajectories. However, dendrites of the DO3–DO5 motor neurons (arrowheads) form anterior to those of the DT1 (arrow in [B]) and the SN motor neurons (arrow in [C]).

Anterior is left and dorsal is up. Symbols and abbreviations: triangles, ventral midline; AC, anterior commissure; PC, posterior commissure; asterisks, dorsoventral channels (landmarks for the segment borders). Scale bar (not applicable to diagrams of CNS and muscle field): 10 μm.

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The Neural Map of the Muscle Field Forms Independently of Its Targets

Since dendritic arbors form after motor axons have reached their targets, the muscles could be instrumental in dictating the organisation of the central map. To test this idea, we used the UAS/GAL4 system (Brand and Perrimon 1993) to misexpress an activated form of Notch (Kidd et al. 1998) in the developing mesoderm, so suppressing the formation of muscle founder cells while leaving other tissues intact (Landgraf et al. 1999). In such muscleless embryos, the main nerve trunks, SN and ISN, still form and project into the periphery (Landgraf et al. 1999). Retrograde labellings of these nerves show that SN and ISN motor neurons form relatively normal dendritic arbors that consistently conform to the characteristic separation of SN and ISN dendrites (Figure 6).

Thus, the neuropile is partitioned into distinct fields of dendritic arborisation independently of the muscles. We conclude that the mapping process is likely to be an autonomous property of the motor neurons and their neighbouring cells.

Glial Cells as Substrates for Dendritic Growth

We next asked whether motor neuron dendritic fields could be patterned by the substrates on which they grow. In the *Drosophila* ventral nerve cord (VNC), motor neuron dendrites form in the dorsal-most region of the neuropile, sandwiched between longitudinal glia above and the underlying scaffold of axons. Glial cells can act as substrates for supporting and guiding axonal growth (Bastiani and Goodman 1986; Hidalgo et al. 1995; Booth et al. 2000). To test whether they might also be required for the growth and spatial patterning of dendritic fields, we analysed dendritic arbors in *glial cells missing* (*gcm*) mutant embryos, which are defective in glial cell differentiation (Hosoya et al. 1995; Jones et al. 1995). Although the structure of the nervous system is disrupted in *gcm* mutant embryos and the dendritic arbors are abnormal, they continue to form in their characteristic locations and the fundamental distinction between the ISN and SN motor neuron dendritic fields is maintained (Figure 7A and 7B). Remarkably, even the long posterior dendritic projection of the DT1 motor neuron forms and reaches its target region, the SN external muscle dendritic domain (Figure 7C and 7D).

These results suggest that the patterning of the neuropile into distinct motor neuron dendritic domains is a process that appears to be intrinsic to the motor neurons and their neighbouring neurons, but does not require proper glial cell differentiation.

Interactions between Dendritic Arbors of Neighbouring Domains

One likely explanation for the division of dendrites into separate domains is that there is a process of mutual exclusion between the arborisations of neighbouring cells. Such a process of dendritic 'tiling' has so far only been documented between particular classes of sensory neurons (Wassle et al. 1981; Grueber et al. 2002, 2003), but could also occur in the motor system. We tested the idea of tiling by considering two groups of motor neurons whose axons have a common trajectory, but whose dendritic fields form in adjacent territories. The DO3–DO5 and DT1 motor neurons

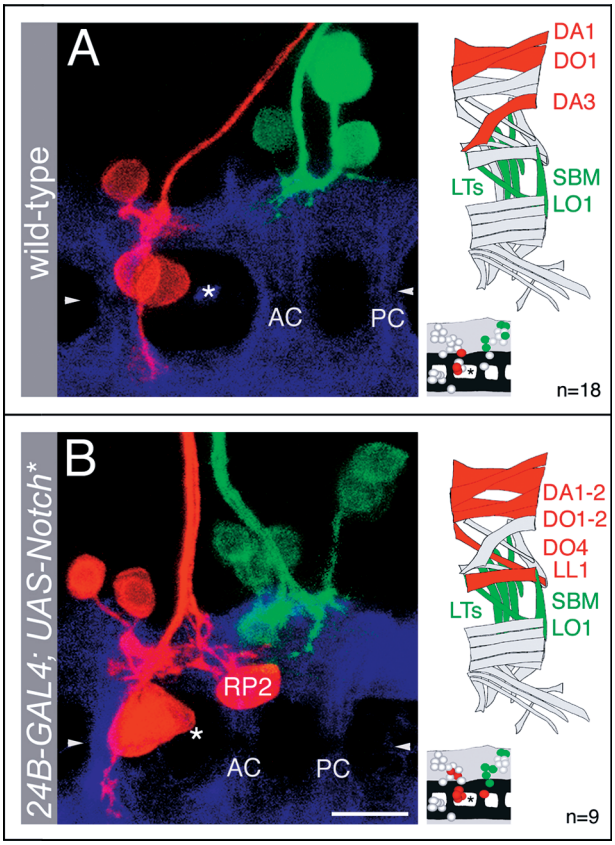

Figure 6. The Myotopic Map Forms Independently of Target Muscles

ISN motor neurons (red) with internal and SN motor neurons (green) with external muscle targets in a 15-h-old wild-type (A) and in an embryo, in which muscle formation had been suppressed by targeted expression of an activated form (intracellular domain) of Notch (*24B-GAL4*; *UAS-Notch**) (B). In such muscleless embryos, the main nerve trunks (ISN and SN) still form and project into the periphery along distinctive paths. Thus, motor neurons whose axons project through these nerves can be retrogradely labelled. The neuropile, visualised with anti-HRP, is shown in blue. ISN and SN motor neuron dendritic domains show a normal separation despite absence of target muscles. Note that the ISN (red) and SN (green) dendritic arbors in (B) appear to be in closer proximity than those shown in (A). This is because in (B) the RP2 neuron (indicated) is labelled, which is the most posterior of the ISN motor neurons and therefore closest to the SN dendritic domain. See also Figure 2G, where RP2 and its dendrites are shown relative to the most posterior of the SN motor neuron (SBM) dendritic fields.

Anterior is left and dorsal is up. Symbols and abbreviations: triangles, ventral midline; AC, anterior commissure; PC, posterior commissure; asterisks, dorsoventral channels (landmarks for the segment borders). Scale bar (not applicable to diagrams of CNS and muscle field): 10 μm.

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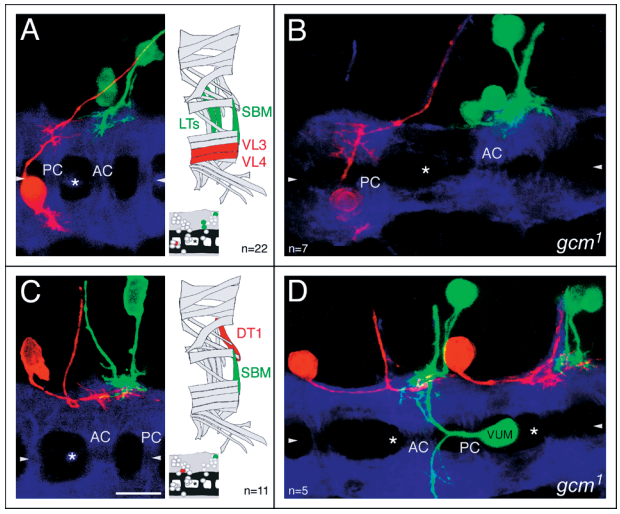

Figure 7. Glial Cell Differentiation Is Not Required for Neuropile Subdivision

ISN (red) and SN (green) motor neurons labelled in 15-h-old wild-type (A and C) and *gcm* mutant (B and D) embryos. The neuropile, visualised with anti-HRP, is shown in blue.

(A and B) Motor neurons innervating ventral (VL3–VL4, RP3) internal (red) and external (green) muscles of a segment elaborate their dendrites in separate regions of the neuropile on either side of the segment border (asterisks). This is accentuated when neuromeres separate in *gcm* mutant embryos (B).

(C and D) The DT1 motor neuron (red) is the only ISN motor neuron whose dendrites branch in the SN dendritic domain (green). This dendritic projection pattern is maintained in *gcm* mutant embryos (D). An SN VUM efferent neuron has also been labelled.

Anterior is left and dorsal is up. Symbols and abbreviations: triangles, ventral midline; AC, anterior commissure; PC, posterior commissure; asterisks, dorsoventral channels (landmarks for the segment borders). Scale bar (not applicable to diagrams of CNS and muscle field): 10 μm.

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project their dendrites posteriorly, and at their most-anterior point, these dendrites meet the axons and dendrites of the anterior corner cell (aCC) and U/CQ neurons (Figure 8A). To show whether the aCC and U/CQ axons and/or dendrites inhibit the growth of DO3–DO5 and DT1 dendrites anteriorly, we selectively ablated these neurons (as well as RP2 and the posterior corner cell [pCC] interneuron) (Fujioka et al. 2003). Using anti-Even-skipped (Eve) staining as a marker for aCC, RP2, and U/CQs (there are an additional two medially located *eve*-expressing interneurons, pCC and friend of pCC [fpCC] [Goodman and Doe 1993; Bossing et al. 1996]), we find that we can efficiently ablate these neurons before they form dendrites (at approximately 11 h AEL): on average, by 10.5 h AEL all but 0.6 (Figure 8B–8C) and by 12 h AEL all but 0.06 of the seven medially located *eve*-expressing neurons have been ablated per half-neuromere ($n = \geq 60$). In no instance did we observe a concomitant anterior expansion of the DO3–DO5 and DT1 motor neuron dendrites into the regions vacated by the aCC and U/CQ dendrites ($n = 13$; Figure 8D). We conclude that, at least in this instance, the initial dendritic territory of one set of motor neurons (DO3–DO5 and DT1) is not defined by a process of tiling, in which they are excluded by neighbouring (aCC and U/CQ) dendritic arbors. However, it is possible that the elaboration of motor neuron dendritic arbors during later developmental stages may involve interactions between neighbouring dendritic territories, activity-dependent processes, or both.

Thus, in summary, our results suggest that the mechanisms that subdivide the neuropile into distinct dendritc domains are very robust and refractory to perturbations. They further suggest that the cues that organise the map may be laid down early in development as the embryo subdivides into parasegmental units.

Discussion

Organisation of Motor Systems

In many sensory systems, the terminals of in-growing axons are organised centrally in a regular fashion to form a map of

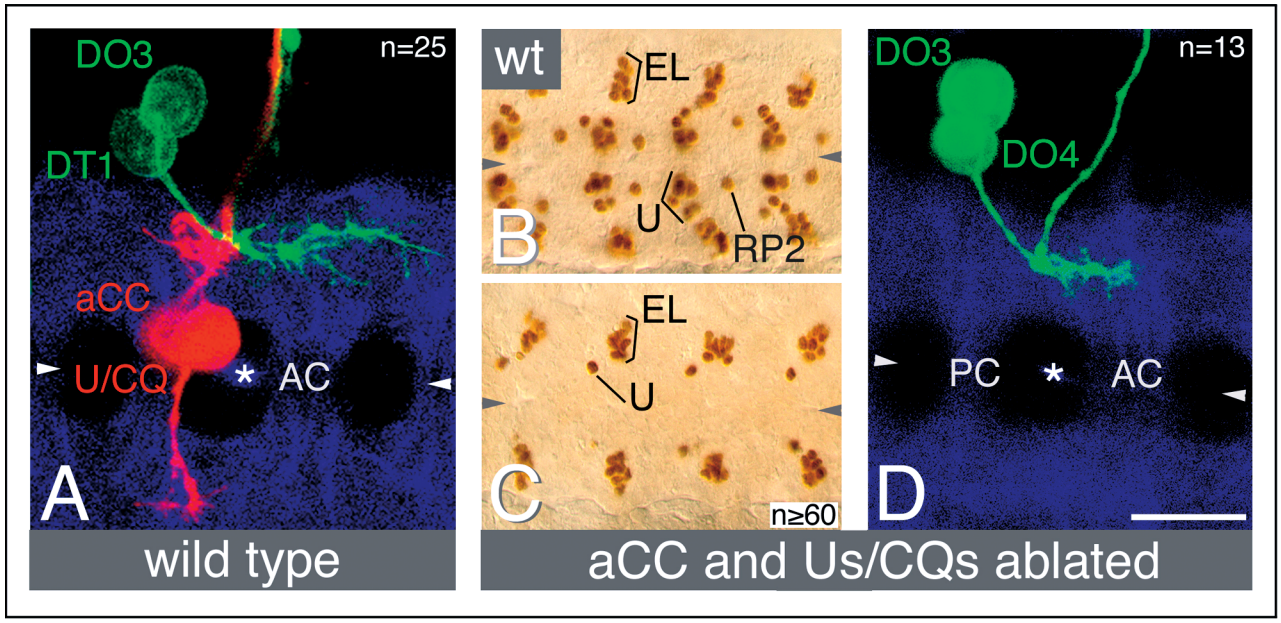

Figure 8. Territories of Initial Dendritic Elaboration Are Not Defined by Mutual Exclusion

(A) Dendrites of DO3 (as well as DO4–DO5 and DT1) (green) motor neurons always project posterior to the region in which the dendritic arbors of the aCC and U/CQ motor neurons (red) form.

(B and C) *eve*-expressing motor neurons (aCC, U/CQs, and RP2) in stage 13 (approximately 10.5-h-old) wild-type embryos (B) and those in which the aCC, RP2, and U/CQ neurons had been selectively ablated (C). In (C), all medial *eve*-expressing neurons have been ablated by this stage, and only one, possibly the U/CQ neuron (likely the LL1 motor neuron), is still present in several segments. U in (B) marks all U/CQ neurons as well as the aCC motor and the pCC and fpCC interneurons contained in this group; EL marks the lateral *eve*-expressing interneurons.

(D) Dendritic arborisations of the DO3–DO4 motor neurons do not elaborate anteriorly into the territory vacated by ablated aCC and U/CQ neurons.

Anterior is left and dorsal is up. Symbols and abbreviations: triangles, ventral midline; AC, anterior commissure; PC, posterior commissure; asterisks, dorsoventral channels (landmarks for the segment borders). Scale bar in (A) and (D): 10 μm; in (B) and (C): 45 μm.

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the periphery within the developing CNS. This organisational principle reduces the problem of understanding the underlying developmental mechanisms by providing a simple anatomical framework within which the growth and targeting of axons can be readily understood. So far, we know a great deal less about the organisation of motor systems, but much of our understanding derives from vertebrate models. In the vertebrate spinal cord, motor neurons with a common target muscle cluster as motor pools, which are grouped to form motor columns (Landmesser 1978). Because there is a topographical relationship between motor pools and their respective target muscles, it has been suggested that the arrangement of motor neuron cell bodies in the spinal cord reflects their functional relationships. Indeed, the anteroposterior positions of motor pools seem to correlate with the proximodistal distribution of limb muscles (Romanes 1951; Cruce 1974). Moreover, motor pools in the medial and lateral subdivisions of the lateral motor column innervate antagonistic ventral and dorsal limb muscles, respectively (Romer 1970; Landmesser 1978; Kania et al. 2000). However, any correlation between motor neuron position and function has to be reconciled with the observation that the arrangement of motor pools in the spinal cord is highly, if not rigidly, conserved among vertebrates in spite of the functional diversification of homologous muscles (Romanes 1951; Cruce 1974; Landmesser 1978). This has prompted suggestions that the arrangement of motor pools might relate to the ontogeny rather than the mature operation of target muscles (Romer 1970; Landmesser 1978). This hypothesis predicts that there are mechanisms by which motor neuron function (as defined by its interneuronal connections) can adapt to evolutionary change in target muscle operation without a shift in cell body position. Thus, the distribution of motor neuron dendrites might be more closely related to actual functional requirements than the arrangements of cell bodies in the spinal cord.

In this paper we have addressed this question using the *Drosophila* neuromuscular system. We also do not find any obvious relationship between motor neuron cell body position (largely a consequence of their birth times and places, as in vertebrates) and function: the segmentally repeated array of body wall muscles is not represented centrally by arrangements of their motor neuron cell bodies (Sink and Whitington 1991). However, we do observe that motor neuron dendrites arborise in different territories of the neuropile and that these correlate precisely with the distributions of their respective target muscles (see Figure 3). Specifically, we find that the muscle field is represented in the neuropile by two principal dendritic domains, which represent distinct muscle sets: the external (transverse) versus the internal (longitudinal) muscles. This is in agreement with Levine and Truman (1985), who suggested that parts of the *Manduca* larval neuromuscular system display a degree of somatotopic organisation, and with earlier work by Burrows (1973) and others, which led to the tentative idea that motor neurons with similar functions emerging through the same nerve trunk have similar shapes. In addition, we find in the motor system of the *Drosophila* larva that within each of the two principal dendritic domains, differences in muscle

position are reflected by corresponding distinctions in the anteroposterior placement of motor neuron dendritic arbors, which together form the myotopic map (see Figures 2 and 3).

Formation of Central Myotopic Representations

Our observations suggest that an important element during nervous system development is the subdivision of the neuropile into functionally distinct domains. We have asked whether this partitioning of the neuropile emerges as a 'passive' consequence of the position of origin and packing of motor neurons. However, the fact that a single NB (NB3-2) can give rise to motor neurons innervating internal muscles in one segment and external muscles in the next suggests that arborisation domains are not a simple reflection of progenitor position. This is reinforced by the converse finding, namely that motor neurons innervating related muscles (e.g., the dorsolateral group) can arise from different NBs (NB3-2 for DO3–DO5, NB7-1 for LL1 and DA3) and have distinct morphologies, yet generate their dendritic arbors in a common region of neuropile (Bossing et al. 1996; Landgraf et al. 1997). In addition, we find that the organisational principle of the myotopic partitioning of the neuropile is confirmed by every atypical motor neuron–muscle pair of the system (including VT1, DT1, and RP2): their territories of dendritic arborisation consistently correlate with the distribution of their respective target muscles rather than their origins.

We find that while the muscle field is segmental in organisation, it is represented centrally by a parasegmental repeat unit of dendritic arborisations, whose boundary is marked by the anterior margin of *en* expression in the CNS. We emphasise here that the boundary between adjacent units is not an absolute one: the parasegmental nature of the repeat is most apparent in the initial domains of dendrite growth, and as the size of arborisations increases, there is overlap between adjacent arbors, though the different positions of dendritic domains remain recognisable (Landgraf et al. 2003). Our observations that the map can form in the absence of target muscles or proper differentiation of glia suggest more autonomous mechanisms at work within the developing CNS. Furthermore, the fact that dendritic partitioning persists despite the loss of any one of six key segment polarity genes tested, which are also required for the formation of a proper pattern of neural progenitor cells, suggests that the machinery of dendritic patterning is a robust feature of the developing nervous system and that it is built in at an early stage, when the elements of the body plan are first laid out as a series of parasegmental units.

Motor System Adaptation

The patterning of the motor neuron dendritic arbors in the *Drosophila* embryo represents a first layer of organisation in the motor system. This is likely in part to be mirrored by the endings of higher-order neurons of central pattern generating circuits, which converge onto the myotopic map. While motor neuron cell body positions may, as has been proposed for vertebrate systems, relate to the ontogeny of target muscles, the operation of mature muscles is reflected by the allegiance of corresponding motor neuron dendrites to a particular territory in the neuropile. Thus, changes in muscle operation could be accommodated by a change of allegiance of the appropriate motor neuron dendrites from one domain to another (e.g., the DT1 motor neuron–muscle pair) without the need for rewiring the underlying higher-order circuitry. Such a model resolves the apparent discrepancy between the distributions of motor neuron cell bodies centrally and target muscles in the periphery. It also implies a considerable degree of flexibility, particularly at the level of motor output, yet suggests that elements of the underlying motor circuitry may have been highly conserved.

Materials and Methods

Fly stocks. The following fly stocks were used: Oregon-R, *en/invected* (*Df(en^{E})*), *wg* (*wg^{CX4}*), *nkd* (*nkd^{2}*), *ptc* (*ptc^{9}*), *hh* (*hh^{21}*) and *gsb* (*Df2R(gsb)*), *gcm^{1}*(Hosoya et al. 1995), *24B-GAL4* (Brand and Perrimon 1993), and *UAS-NotchIntra* (activated form of Notch) (Kidd et al. 1998). aCC, pCC, RP2, and U/CQ neurons were ablated using *RN2-GAL4;CQ-GAL4* (see below) crossed to *w;tubulin>CD2>GAL4,UAS-FLP1.D;UAS-reaper/SM5-TM6b* (Hidalgo and Brand 1997; Pignoni and Zipursky 1997).

Cell labellings. Embryos 15 h old were dissected as described in Landgraf et al. (1997) (without collagenase treatment), and embryos 19 h old were dissected as described in Baines and Bate (1998). Retrograde labellings were as described by Landgraf et al. (1997), and in addition neuromuscular junctions were visualised by Cy2-conjugated anti-horseradish peroxidase (HRP) incubation (Jackson ImmunoResearch, West Grove, Pennsylvania, United States; 1:50 dilution), followed by saline washes; DiD (Molecular Probes, Eugene, Oregon, United States) was used at 2 mg/ml in oil. In segment polarity mutants (see Figure 4) and in muscleless embryos (see Figure 6), SN neurons were labelled by iontophoretic DiI application to the SN as described by Sink and Whitington (1991). Anterograde labellings were done as in Zlatic et al. (2003).

Immunocytochemistry. Primary antibodies were anti-Lucifer Yellow (1:1000 dilution; Molecular Probes), anti-Eve (1:5000 dilution; gift from M. Frasch [Frasch et al. 1987]), and Cy2-conjugated anti-HRP (1:100 dilution; Jackson ImmunoResearch). Secondary antibodies were Alexa488-conjugated antirabbit (1:500 dilution; Molecular Probes) and biotinylated antirabbit (1:500 dilution). Standard methods were followed (Patel 1994), using 0.3% Triton X-100 as a detergent for anti-Lucifer Yellow diaminobenzidine stainings. Immunofluorescence was visualised with a Leica SP confocal microscope (Leica Microsystems, Wetzlar, Germany). All images shown are maximum projections of confocal z-series that were processed using Adobe Photoshop (Adobe Systems Incorporated, San Jose, California, United States).

Plasmid construction. The Gal4 drivers RN2-Gal4 and CQ2-Gal4 were constructed as followed. The *eve* 5′-promoter region from −275 (*Sfi*I) to +11 bp (*Xho*I) was fused to a 38 bp multicloning sequence upstream of *eve* DNA from +91 to +99 nt, followed by an ATG of the Gal4-coding sequence, replacing the yeast *GAL4* translation initiation signal with that of *eve*. The Gal4-coding region from plasmid pCEP4-Gal4 (gift from S. Thor) was followed by the *eve* 3′ region from +1306 to +1521 (*Kpn*I). For the RP2+a/pCC-specific Gal4 drivers (*RN2-GAL4*), two tandem repeats of the RP2+a/pCC element from +7.9 (*Eco*RI) to +8.6 kb (*Nhe*I) were placed upstream of the *eve* 5′ promoter and Gal4-coding region (Fujioka et al. 2003). For the U/CQ-specific Gal4 drivers (*CQ2-GAL4*), two tandem repeats of the U/CQ neuronal element from +3.5 (*Bgl*II)to +4.3 kb (*Msc*I) were placed upstream of the same promoter-Gal4-coding region. Transgenic lines were established as described previously (Rubin and Spradling 1982; Fujioka et al. 2000).

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Conflicts of Interest. The authors have declared that no conflicts of interest exist.

Author Contributions. ML and MB conceived and designed the experiments. ML and VJ performed the experiments. ML and VJ analysed the data. MF and JBJ contributed reagents/materials/analysis tools. ML and MB wrote the paper. ∎

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